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PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Nunomura	Group Art Unit Unknown
Serial No.:	Unknown Continuation of App. No. 09/620,958	
Filed:	Herewith	
For:	POLYNUCLEOTIDE AMPLIFICATION METHOD	
Examiner:	Unknown	

PRELIMINARY AMENDMENT

Box Patent Application
Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Preliminary to examination on the merits, please amend the above-captioned continuation application as follows:

IN THE SPECIFICATION:

On page 1 of the Specification, after the Title of the Invention ending on line 2 and before the Field of the Invention on line 4, please insert --This application is a continuation of Application No. 09/620,958, filed July 21, 2000, which claims the benefit of U.S. Provisional Application No. 60/145,432, filed July 23, 1999.--

IN THE CLAIMS:

Please cancel Claims 1-55 without prejudice.

Please add the following new claims:

56. A method of quantifying analyte polynucleotides present in a test sample, comprising the steps of:

obtaining a test sample that contains an unknown amount of an analyte polynucleotide, said analyte polynucleotide being selected from the group consisting of a viral polynucleotide, a bacterial polynucleotide, a fungal polynucleotide, a protozoan polynucleotide, and a human polynucleotide;

combining a predetermined amount of said test sample with a predetermined amount of a pseudo target;

co-amplifying in a polynucleotide amplification reaction the pseudo target and any of the analyte polynucleotide contained in said test sample to produce a collection of amplification products, said collection including both an analyte amplicon if said test sample contained the analyte polynucleotide and a pseudo target amplicon; and

quantifying the analyte amplicon without reference to the amount of pseudo target amplicon, whereby the quantity of analyte amplicon is related in a manner that is dose-dependent on the unknown amount of the analyte polynucleotide contained in said test sample.

57. The method of Claim 56, further comprising a step for detecting the pseudo target amplicon.

58. The method of Claim 56, wherein the step for quantifying comprises hybridizing said collection of amplification products from the co-amplifying step with a labeled probe specific for the analyte amplicon but not the pseudo target amplicon and then detecting any labeled probe that specifically hybridized the analyte amplicon.

59. The method of Claim 58, wherein said polynucleotide amplification reaction in the co-amplifying step is selected from the group consisting of a Transcription Mediated Amplification reaction, a NASBA reaction and a Polymerase Chain Reaction.

60. The method of Claim 59, wherein said polynucleotide amplification reaction is the Transcription Mediated Amplification reaction.

61. The method of either Claim 59 or Claim 60, wherein the obtaining step comprises first collecting a biological specimen and then releasing nucleic acids contained therein to result in said test sample that contains the unknown amount of said analyte polynucleotide.

62. The method of Claim 61, further comprising a step for capturing said analyte polynucleotide onto a solid support prior to said co-amplifying step.

63. The method of Claim 62, wherein the solid support is a bead derivatized with a synthetic polynucleotide.

64. The method of Claim 62, wherein the predetermined amount of said pseudo target in the combining step ranges from between 1.0×10^3 and 2×10^8 molecules.

65. The method of Claim 64, wherein the predetermined amount of said pseudo target in the combining step ranges from between 1.0×10^4 and 2×10^8 molecules.

66. The method of Claim 65, wherein the predetermined amount of said pseudo target in the combining step ranges from between 1.0×10^5 and 2×10^8 molecules.

67. The method of Claim 61, wherein the biological specimen is a blood sample or a plasma sample and wherein said nucleic acids comprise viral nucleic acids.

68. The method of Claim 67, wherein the analyte polynucleotide is released from HIV virions.

69. The method of Claim 61, wherein the predetermined amount of the pseudo target in the combining step is between 1×10^3 and 2×10^8 molecules.

70. The method of Claim 69, wherein the predetermined amount of the pseudo target in the combining step is between 1×10^4 and 2×10^8 molecules.

71. The method of Claim 70, wherein the predetermined amount of the pseudo target in the combining step is between 1×10^5 and 2×10^8 molecules.

72. The method of Claim 60, further comprising a step for isolating said analyte polynucleotide and said pseudo target after the combining step and before the co-amplifying step.

73. The method of Claim 60, wherein the predetermined amount of the pseudo target is between 1×10^3 and 2×10^8 molecules.

74. The method of Claim 73, wherein the predetermined amount of the pseudo target is between 1 x 10^4 and 2 x 10^8 molecules.

75. The method of Claim 74, wherein the predetermined amount of the pseudo target is between 1 x 10^5 and 2 x 10^8 molecules.

76. The method of Claim 58, wherein said labeled probe is labeled with acridinium ester.

77. The method of Claim 76, wherein the quantifying step comprises measuring by luminometry the probe labeled with acridinium ester that specifically hybridized the analyte amplicon.

78. The method of Claim 61, wherein the analyte polynucleotide is a viral polynucleotide.

79. The method of Claim 56, further comprising a step for consulting a standard curve that relates pre-amplification amounts of analyte polynucleotide and post-amplification amounts of analyte amplicon.

80. The method of Claim 60, further comprising a step for consulting a standard curve that relates pre-amplification amounts of analyte polynucleotide and post-amplification amounts of analyte amplicon.

81. The method of Claim 77, further comprising a step for consulting a standard curve that relates pre-amplification amounts of analyte polynucleotide and post-amplification amounts of analyte amplicon.

82. The method of Claim 60, wherein the Transcription Mediated Amplification reaction employs a paired set of oligonucleotide primers have the sequences of SEQ ID NO:1 and SEQ ID NO:2.

83. The method of Claim 82, wherein the pseudo target has a polynucleotide sequence selected from the group consisting of SEQ ID NO:4 and SEQ ID NO:9.

84. A method for relating pre-amplification amounts of analyte polynucleotide and post-amplification amounts of analyte amplicon, said method comprising the steps of:

obtaining a plurality of control samples, wherein each of the control samples has a different predetermined amount of an analyte polynucleotide, said analyte polynucleotide being selected from the group consisting of a viral polynucleotide, a bacterial polynucleotide, a fungal polynucleotide, a protozoan polynucleotide, and a human polynucleotide;

combining each of said plurality of control samples with a constant predetermined amount of a pseudo target to result in a plurality of mixed control samples;

co-amplifying in a plurality of amplification reactions both the pseudo target and the analyte polynucleotide present in each of said plurality of mixed control samples to produce a collection of amplification products that include a pseudo target amplicon and an analyte amplicon;

quantifying the analyte amplicon for each of said plurality of amplification reactions without reference to the amount of pseudo target amplicon present in said collection of amplification products; and

preparing a standard curve having the different predetermined amounts of said analyte polynucleotide plotted against the quantified amounts of said analyte amplicon produced in each of said plurality of amplification reactions, thereby relating the pre-amplification amounts of said analyte polynucleotide in said plurality of control samples and the post-amplification amounts of analyte amplicon synthesized in the plurality of amplification reactions.

85. The method of Claim 84, further comprising a step for detecting the pseudo target amplicon.

86. The method of Claim 84, wherein said analyte polynucleotide is a viral polynucleotide.

87. The method of Claim 86, wherein the viral polynucleotide is an HIV polynucleotide.

88. The method of Claim 84, wherein said constant predetermined amount of said pseudo target is between 1×10^3 and 2×10^8 molecules.

89. The method of Claim 88, wherein said constant predetermined amount of said pseudo target is between 1 x 10^4 and 2 x 10^8 molecules.

90. The method of Claim 89, wherein said constant predetermined amount of said pseudo target is between 1 x 10^5 and 2 x 10^8 molecules.

91. The method of Claim 84, wherein said plurality of amplification reactions in the co-amplifying step is selected from the group consisting of a plurality of Transcription Mediated Amplification reactions, a plurality of NASBA reactions and a plurality of PCR reactions.

92. The method of Claim 91, wherein said plurality of amplification reactions in the co-amplifying step are a plurality of Transcription Mediated Amplification reactions.

93. The method of Claim 91 or Claim 92, wherein the quantifying step comprises hybridizing said collection of amplification products from the co-amplifying step with a labeled probe specific for the analyte amplicon but not the pseudo target amplicon and then quantitatively detecting any labeled probe that specifically hybridized the analyte amplicon.

94. The method of Claim 93, wherein the labeled probe is labeled with acridinium ester.

95. The method of Claim 93, further comprising a step for capturing said analyte polynucleotide onto a solid support prior to said co-amplifying step.

96. A method of determining whether a biological sample contains an analyte polynucleotide, comprising the steps of:

obtaining a biological sample to be tested for the presence of the analyte polynucleotide, said analyte polynucleotide being selected from the group consisting of a viral polynucleotide, a bacterial polynucleotide, a fungal polynucleotide, a protozoan polynucleotide, and a human polynucleotide;

combining the biological sample with a pseudo target to result in a mixed sample;

isolating nucleic acids from the mixed sample, whereby there is obtained a collection of molecules comprising the pseudo target and any of said analyte polynucleotide present in the biological sample;

conducting a polynucleotide amplification reaction to co-amplify the pseudo target and any of said analyte polynucleotide contained in said collection of molecules to produce amplification products, whereby pseudo target amplicons are formed, and whereby analyte amplicons are formed if said collection of molecules included said analyte polynucleotide;

detecting in said amplification products any of said analyte amplicons without detecting said pseudo target amplicons; and

determining that the biological sample contains said analyte polynucleotide if said analyte amplicons are detected in the amplification products.

97. The method of Claim 96, wherein the amplification reaction is selected from the group consisting of a Transcription Mediated Amplification reaction, a NASBA reaction and a PCR reaction.

98. The method of Claim 97, wherein the amplification reaction is a Transcription Mediated Amplification reaction.

99. The method of Claim 98, wherein the obtaining step comprises drawing blood.

100. The method of either Claim 97 or Claim 98, wherein the detecting step comprises first hybridizing a labeled polynucleotide probe having binding specificity for the analyte amplicons and then measuring the extent of specific binding of the labeled polynucleotide probe and the analyte amplicons.

101. The method of Claim 100, wherein the isolating step comprises immobilizing said pseudo target and said analyte polynucleotide to a solid support.

102. The method of Claim 100, wherein the detecting step comprises detecting by luminometry.

103. The method of Claim 102, wherein the analyte polynucleotide is from HIV virions.

104. The method of Claim 103, wherein the pseudo target has a sequence selected from the group consisting of SEQ ID NO:4 and SEQ ID NO:9.

105. A method of determining whether an analyte polynucleotide is present in a test sample in an amount greater or less than a pre-determined value, comprising the steps of:

obtaining a test sample to be analyzed for the presence of said analyte polynucleotide, said analyte polynucleotide being selected from the group consisting of a viral polynucleotide, a bacterial polynucleotide, a fungal polynucleotide, a protozoan polynucleotide, and a human polynucleotide;

combining said test sample with an amount of a pseudo target;

co-amplifying in a polynucleotide amplification reaction the pseudo target and any analyte polynucleotide contained in said test sample to produce amplification products that include a pseudo target amplicon and an analyte amplicon, wherein said analyte amplicon is present in an amount that is dose-dependent on the amount of said analyte polynucleotide present in said test sample; and

quantitatively detecting said analyte amplicon using a detection system calibrated to have a detection threshold corresponding to a signal strength arising from co-amplification of said amount of said pseudo target and an amount of analyte polynucleotide corresponding to said pre-determined value, wherein detection of a signal above or below said threshold of detection indicates that said analyte polynucleotide is present in said test sample in an amount that is respectively greater or less than said pre-determined value.

106. The method of Claim 105, further comprising a step for detecting the pseudo target amplicon produced in the co-amplifying step.

107. The method of Claim 105, wherein the amount of said pseudo target in the combining step may be increased or decreased to change the detection threshold of said detection system.

108. The method of Claim 105, wherein said detection system comprises luminometry.

109. The method of Claim 105, wherein said analyte polynucleotide is a viral polynucleotide.

110. The method of Claim 109, wherein said viral polynucleotide is selected from the group consisting of an HIV-1 polynucleotide, an HIV-2 polynucleotide, an HBV polynucleotide, and an HCV polynucleotide.

111. A kit for performing a polynucleotide amplification reaction using an analyte polynucleotide as a template, comprising:

a pseudo target;

at least one pair of oligonucleotide primers for co-amplifying the pseudo target and the analyte polynucleotide, said analyte polynucleotide being selected from the group consisting of a viral polynucleotide, a bacterial polynucleotide, a fungal polynucleotide, a protozoan polynucleotide, and a human polynucleotide;

reagents for carrying out the polynucleotide amplification reaction, said reagents including deoxynucleotide triphosphates and a DNA polymerizing enzyme; and

printed instructions with directions for first carrying out the amplification reaction and then detecting only analyte amplicons produced in the amplification reaction.

112. The kit of Claim 111, further comprising a labeled probe for detecting any analyte amplicons produced in the amplification reaction.

113. The kit of Claim 111, wherein said reagents further include nucleotide triphosphates and an RNA polymerizing enzyme.

114. The kit of Claim 113, wherein the DNA polymerizing enzyme is a reverse transcriptase.

115. The kit of Claim 114, wherein no RNase H additional to that provided by said reverse transcriptase is used.

REMARKS

Claims 56-115 are presented for examination. Original Claims 1-55 have been canceled and Claims 56-115 have been added to more precisely define the invention of this continuation application. More particularly, the claims in the present application differ from those in the allowed parent application by listing particular source organisms for the recited analyte

polynucleotides. Support for the claims can be found in the specification on page 31 at lines 7-30.

The Specification has been amended to indicate that this application claims domestic priority to co-pending U.S. Application No. 09/620,958.

No new matter is being added by this Preliminary Amendment.

Should there be any questions concerning this application, the Examiner is respectfully invited to contact the undersigned at the telephone number appearing below.

Respectfully submitted,

GEN-PROBE INCORPORATED

Dated: August 30, 2001

By: _____

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